Optically Active Selenium-Containing Amino Acids. The Synthesis of L-Selenocystine and L-Selenolanthionine[1-3]

J. ROY, WOLFGANG GORDON, I. L. SCHWARTZ, AND RODERICH WALTER

The Mount Sinai Medical and Graduate Schools of The City University of New York, New York, New York 10029, and The Medical Research Center, Brookhaven National Laboratory, Upton, New York 11973

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During the past few years, increased attention has been given to biologically active selenocysteine-containing peptides.[4-8] In this context it was important to develop synthetic methods which would permit the introduction of selenium into optically active amino acids. Recently, we described a convenient procedure for the preparation of Se-benzyl-L-selenocysteine derivatives. This method, consisting of a nucleophilic displacement of the O-*p*-toluenesulfonate moiety of an O-tosylated L-serine derivative by the benzyl selenolate anion, is advantageous for the preparation of Se-benzyl-L-selenocysteine compounds which bear selectively removable amino- and carboxyl-protecting groups.[7,8]

In order to demonstrate the wider scope for this displacement reaction in the synthesis of selenium-containing amino acids, we investigated the possibility of transforming an O-tosylated L-serine derivative with sodium hydrogen selenide to the corresponding selenocysteine derivative. Such a derivative with its free selenol function would provide a key intermediate, allowing the transformation either to the diselenide by oxidation or to selenides by alkylation. While the former type of reaction would pave the way for a convenient synthesis of L-selenocystine, the latter would offer a route toward the synthesis of dialkyl-selenides which possess a selenocysteine moiety as the basic skeleton, such as L-selenolanthionine. The latter reaction path would also permit the introduction of selectively removable selenium-protecting groups.

To examine the feasibilty of the above concept, N-carbobenzoxy-O-tosyl-L-serine diphenylmethyl ester (I)[8] was allowed to react with a stoichiometric amount of sodium hydrogen selenide. In view of the ease with which aliphatic selenols oxidize,[9,10] we did not attempt to isolate the N-carbobenzoxy-L-selenocysteine diphenylmethyl ester but instead converted the selenol *in situ* into the corresponding diselenide, bis(diphenylmethyl)bis(N-carbobenzoxy)-L-selenocystinate (IIa). Although the reaction mixture gave a solid corresponding to 85% yield, various preparations of IIa gave different optical rotations, and a close examination of the reaction product on tlc revealed two compounds which exhibit almost identical R_f values. Repeated slow crystallizations of the reaction mixture finally gave IIa in moderate yield with only a trace of by-product. The preparation of II in high yield, completely free of contaminants, and with reproducible optical activity was achieved by the following route. N-Carbobenzoxy-Se-benzoyl-L-selenocysteine diphenylmethyl ester (III)—readily secured by the acylation of N-carbobenzoxy-L-selenocysteine diphenylmethyl ester—was debenzoylated with hydroxylamine and oxidized *in situ* to yield II. The ester II was cleaved with 0.9 *N* hydrogen chloride in nitromethane[11] and the reaction product was isolated as the crystalline bisdicyclohexylammonium salt (IV). The nitrogen function of the free acid of IV was liberated by treatment with hydrogen bromide in glacial acetic acid, and, upon adjustment of the solution of the hydrobromide to pH 5, the L-selenocystine (V) was secured (Scheme I). In addition, L-selenocystine was prepared by another method. While the above route, starting with the fully protected selenocysteine derivative III, proceeded according to the scheme (a) liberation of selenium moiety, (b) oxidative dimerization, (c) liberation of carboxyl group, and (d) liberation of nitrogen moiety, the route described below entails the following steps: (a) liberation of nitrogen and carboxyl moieties, (b) liberation of selenium moiety, and (c) oxidative dimerization. For this purpose the sodium salt of N-carbobenzoxy-L-selenocysteine diphenylmethyl ester was allowed to react with diphenylmethyl bromide to yield N-carbobenzoxy-Se-diphenylmethyl-L-selenocysteine diphenylmethyl ester (VI). Apparently, this alkylation proceeds slowly and a prolonged reaction time is required to obtain VI in high yield. If the reaction time is shortened, II, formed from the unreacted selenol during the work-up procedure of the reaction, is isolated as a major by-product. When diphenylmethyl toluene-*p*-sulfonate,[12] instead of the diphenylmethyl bromide, is used for alkylation, the reaction does not proceed any faster. Decarbobenzoxylation and simultaneous deesterification of VI were achieved by treatment with HBr–AcOH and the resulting Se-diphenylmethyl-L-selenocysteine on reaction with TFA and oxidation *in situ* gave V.

Horn, *et al.*, described the isolation of the sulfur-containing amino acid lanthionine [bis(β-amino-β-carboxyethyl)sulfide] from wool[13] and from several proteins[14,15] after being treated with alkalies. More recently, it has come to light that L-lanthionine may be a

(1) This work was supported by U. S. Public Health Service Grant AM-10080 of the National Institute of Arthritis and Metabolic Diseases and by the U. S. Atomic Energy Commission.

(2) The following abbreviations have been adopted: Z = $C_6H_5CH_2OCO$; Ts = $H_3CC_6H_4SO_2$; DMFA = N,N-dimethylformamide; AcOH = acetic acid; EtOH = ethanol; MeOH = methanol; Et_2O = diethyl ether; EtOAc = ethyl acetate; TFA = trifluoroacetic acid.

(3) (a) This work was presented in part before the First American Peptide Symposium at Yale University, Conn., Aug 1968 (R. Walter and M. Dekker, New York, in press). (b) Since the completion of this work, the synthesis of L-selenolanthionine by an independent route has been reported: G. Zdansky, *Ark. Kemi*, **29**, 443 (1968).

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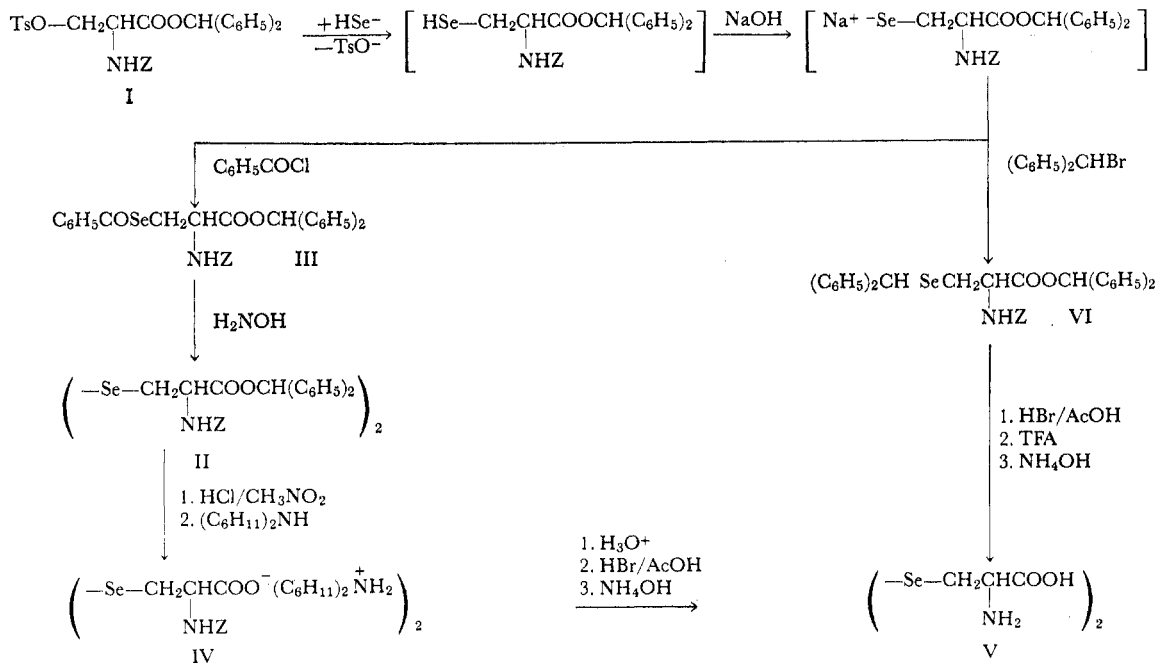
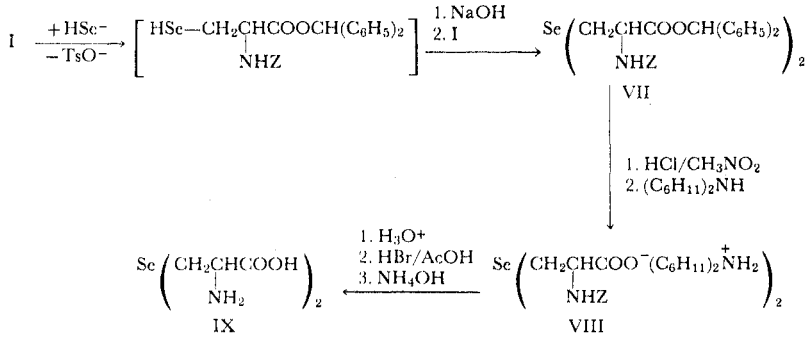
SCHEME I



SCHEME II



naturally occurring amino acid.[16,17] We therefore set out to synthesize the optically active seleno isolog as a further test of our method. The experimental path followed is outlined in Scheme II. The O-tosylated ester I was allowed to react with sodium hydrogen selenide and converted into the selenolate, which in turn was alkylated by addition of a second mole of I. The resulting protected L-selenolanthionine (VII) was hydrolyzed and the bis(N-carbobenzoxy)-L-selenolanthionine was isolated as the crystalline dicyclohexylammonium salt (VIII). The hydrobromide salt, obtained after decarbobenzoxylation of the acid liberated from VIII, was converted into the free base of L-selenolanthionine (IX).

Experimental Section[18]

Preliminary Preparation of Bis(diphenylmethyl)bis(N-carbobenzoxy)-L-selenocystinate (IIa).—Under a hydrogen atmosphere, sodium (0.075 g) was dissolved in EtOH (5 ml) and the resulting mixture was saturated with hydrogen selenide. To this solution, I (1.25 g) dissolved in degassed DMFA (2 ml) was added and the reaction mixture was stirred for an additional 20 min. After the addition of EtOAc (80 ml), the organic phase was extracted with water (three 20-ml portions). The organic phase was separated, dried, and evaporated. The residue crystallized from EtOAc–petroleum ether (bp 30–60°): yield 0.895 g (85%); mp 90–93°; $[\alpha]^{23}D$ −57.4° (2% in DMFA). Examination on tlc with C_6H_6–EtOAc (9:1, v/v) as solvent system revealed two compounds with very close R_f values. Repeated recrystallizations from EtOAc–EtOH gave an almost pure product: yield 0.65 g (62%); mp 96–98°; $[\alpha]^{24}D$ −82.2° (2% in DMFA).

N-Carbobenzoxy-Se-benzoyl-L-selenocysteine Diphenylmethyl Ester (III).—Sodium hydrogen selenide was prepared from sodium (0.065 g) dissolved in absolute EtOH (5 ml) as described above. To the resulting solution, I (1.12 g), which was dissolved in degassed DMFA (2 ml) was added and the reaction mixture was stirred for an additional 30 min. After this period, NaOH (0.1 g) dissolved in degassed water (1 ml) was added, followed by benzoyl chloride (1.13 g) in degassed DMFA (3 ml). The exothermic reaction mixture was stirred under hydrogen inflow for 45 min and then diluted with EtOAc (80 ml). The organic phase was separated and washed with water (three 25-ml portions), dried, and evaporated. The residue was thoroughly extracted with petroleum ether and the remaining solid was crystallized from absolute EtOH: yield 0.9 g (78.3%); mp 82–84°; $[\alpha]^{22}D$ −42.0° (2% in DMFA).

Anal. Calcd for $C_{31}H_{27}NO_5Se$: C, 65.0; H, 4.76; N, 2.45. Found: C, 64.8; H, 4.64; N, 2.40.

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Final Preparation of Bis(diphenylmethyl)bis(N-carbobenzoxy)-L-selenocystinate (II).—Sodium (0.0144 g) was dissolved in anhydrous MeOH (2 ml); to this solution hydroxylamine hydrochloride (0.0434 g) dissolved in MeOH (2 ml) was added. To the stirred reaction mixture N-carbobenzoxy-Se-benzoyl-L-selenocysteine diphenylmethyl ester (0.286 g) in DMFA (2 ml) was added. After 75 min of continued stirring, the light yellow solution was diluted with 75 ml of EtOAc. The organic phase was separated and washed with water (two 25-ml portions) and dried; removal of the solvent gave an oil. Fine, faint yellow needles were obtained by crystallization from MeOH: yield 0.19 g (81%); mp 101–102°; $[\alpha]^{24}D$ −85.2° (2% in DMFA).

Anal. Calcd for $C_{48}H_{44}N_2O_8Se_2$: C, 61.7; H, 4.74; N, 3.00. Found: C, 61.8; H, 4.62; N, 2.93.

Bis(dicyclohexylammonium)bis(N-carbobenzoxy)-L-selenocystinate (IV).—The ester II (0.6 g), dissolved in a solution of 0.9 *N* HCl in nitromethane (10 ml), was kept for 1 hr at room temperature. After this period, thin layer chromatography [C_6H_6–EtOAc (9:1)] revealed the absence of starting material. The nitromethane was evaporated and the oily residue was thoroughly extracted with dilute $NaHCO_3$ solution. The aqueous phase was extracted with Et_2O (two 25-ml portions), separated, and, after acidification with 2 *N* H_2SO_4, extracted with EtOAc (three 20-ml portions). The organic solution was washed two times with small amounts of H_2O, dried with Na_2SO_4, and evaporated. The syrupy residue (0.39 g) was dissolved in MeOH (3 ml), and dicyclohexylamine (0.3 g) dissolved in MeOH (1 ml) was added. Upon addition of Et_2O, a crystalline precipitate resulted, which was collected and recrystallized from MeOH–Et_2O: yield 0.6 g (97%); mp 177–178°; $[\alpha]^{22}D$ −49.5° (1% in MeOH).

Anal. Calcd for $C_{46}H_{70}N_4O_8Se_2$: C, 57.3; H, 7.31; N, 5.81. Found: C, 57.4; H, 7.39; N, 5.71.

L-Selenocystine (V).—The salt IV (0.4 g) was dissolved in a mixture of 1 *N* H_2SO_4 (30 ml) and EtOAc (30 ml). The organic phase was separated, washed three times with a few milliliters of 2 *N* H_2SO_4 and three times with a few milliliters of H_2O, dried over anhydrous Na_2SO_4, and evaporated, and the residue was dried over P_2O_5. The resulting oil was dissolved in dry AcOH (1 ml) and 4 *N* HBr in AcOH (1 ml) was added. During the next 30 min, while the mixture was allowed to stand at room temperature, the HBr salt precipitated partially. Precipitation was completed by addition of Et_2O and the solid material was filtered and repeatedly washed with Et_2O. After being dried under vacuum over KOH and P_2O_5, the HBr salt was dissolved in H_2O (2 ml) and the product was precipitated by adjusting the pH to 5 with dropwise addition of 2 *N* NH_4OH. The canary yellow product was washed with H_2O (2 ml) and dried over P_2O_5 under vacuum: yield 0.125 g (90%); mp 218° dec; $[\alpha]^{25}D$ −162° (1% in 2 *N* HCl) {lit.[19] mp 215° dec; $[\alpha]^{25}D$ −162° (2 *N* HCl)}; ir 2080 (NH_3^+), 1620, and 1580 cm^{-1} (carboxylate).

Anal. Calcd for $C_6H_{12}N_2O_4Se_2$: C, 21.5; H, 3.85; N, 8.36. Found: C, 21.6; H, 3.68; N, 8.52.

Upon amino acid analysis, L-selenocystine emerged at 156.8 ml after the start of the chromatogram; an identical value for the time of emergence was found previously.[20] Glycine, which emerged at 105.6 ml, served as a position marker.

N-Carbobenzoxy-Se-diphenylmethyl-L-selenocysteine Diphenylmethyl Ester (VI).—To the sodium salt of N-carbobenzoxy-L-selenocysteine diphenylmethyl ester prepared from 1.12 g of I (as detailed for the preparation of III) was added 1.0 g of diphenylmethyl bromide dissolved in 2 ml of degassed DMFA. The reaction mixture was stirred under hydrogen for 3 hr and then the tightly stoppered reaction flask was left in the dark for 72 hr at room temperature. The reaction mixture was diluted with EtOAc (100 ml) and washed with water (three 25-ml portions); the organic phase was separated, dried, and evaporated. The resulting oil was chromatographed on a silica gel column; unreacted diphenylmethyl bromide was eluted with C_6H_6 and the product with C_6H_6–EtOAc (99:1, v/v). The fractions containing the product were combined and evaporated, and the resulting oily residue was crystallized from EtOH: yield 1.0 g (79%); mp 104–104.5°; $[\alpha]^{24}D$ −36.5° (2% in DMFA).

Anal. Calcd for $C_{37}H_{33}NO_4Se$: C, 70.0; H, 5.24; N, 2.21. Found: C, 69.7; H, 5.44; N, 2.32.

Alternative Preparation of L-Selenocystine.—To a solution of VI (0.3 g) in dry AcOH (1 ml), 4 *N* HBr in AcOH (1 ml) was added. After 1 hr, the reaction mixture was evaporated and the resulting white solid material was triturated repeatedly with dry Et_2O and then with a few drops of water, yield 0.1 g (63%), mp 149–150°. The infrared spectrum taken in KBr [3020 and 3040 (phenyl stretching), 1620 and 1580 (carboxylate), and 700, and 750 cm^{-1} (phenyl bending)] indicated that the Se-diphenylmethyl-L-selenocysteine was isolated as its zwitterion rather than as the hydrobromide. This compound was suspended in TFA (3 ml) and after addition of phenol (0.4 g) the reaction mixture was heated under reflux for 20 min. The volatile solvent was evaporated under reduced pressure to dryness; the residual liquid was dissolved in water (2 ml) and washed with Et_2O (two 10-ml portions); and the pH was then adjusted to 5 with 2 *N* NH_4OH when a yellow solid was obtained, yield 0.04 g (80%), which had identical physical properties as V described above.

Bis(diphenylmethyl)bis(N-carbobenzoxy)-L-selenolanthionate (VII).—Into absolute EtOH (4 ml) containing sodium (0.055 g) H_2Se was bubbled. Into the solution, I (0.8 g) dissolved in DMFA (2 ml) was introduced. After 10 min of stirring, NaOH (0.068 g) dissolved in H_2O (1 ml) was added followed by I (0.80 g) in DMFA (2 ml). The mixture was stirred for an additional 20 min. The reaction mixture was extracted with EtOAc (50 ml) which was washed with H_2O (one 40-ml and two 10-ml portions), dried, and evaporated. The resulting residue was crystallized from EtOAc–petroleum ether: yield 0.752 g (61.5%); mp 114°; $[\alpha]^{22}D$ −31.7° (1% in DMFA); ir 3335 (NH), 1740 (ester), and 1690 cm^{-1} (urethane).

Anal. Calcd for $C_{48}H_{44}N_2O_8Se$: C, 67.4; H, 5.18; N, 3.27. Found: C, 67.6; H, 5.30; N, 3.19.

Bis(dicyclohexylammonium)bis(N-carbobenzoxy)-L-selenolanthionate (VIII).—A solution of 0.9 *N* HCl in nitromethane (20 ml) containing VII (0.665 g) was kept for 1.5 hr at 25°; then the solvent was evaporated under reduced pressure. The resulting residue was taken up in petroleum ether (15 ml) which was then extracted with half-concentrated $NaHCO_3$ solution (20 ml). The aqueous phase was acidified with 2 *N* H_2SO_4 and extracted with EtOAc (three 20-ml portions). After the combined organic fractions were washed with H_2O (four 10-ml portions), the solution was dried and the solvent was evaporated. Upon dilution of the oily residue (0.381 g) with MeOH (2 ml) and addition of dicyclohexylamine (0.334 g) and Et_2O, a crystalline product resulted which was collected and recrystallized from MeOH–Et_2O: yield 0.61 g (93%); mp 172–173°; $[\alpha]^{22}D$ −0.88° (1% in MeOH); ir 3400 and 3250 (NH), 1715 (urethan), and 1635 cm^{-1} (carboxylate).

Anal. Calcd for $C_{46}H_{70}N_4O_8Se$: C, 62.4; H, 7.96; N, 6.32. Found: C, 62.6; H, 8.08; N, 6.32.

L-Selenolanthionine (IX).—Compound VIII (0.310 g) was dissolved by shaking with a mixture of 2 *N* H_2SO_4 (40 ml) and EtOAc (40 ml). The organic phase was separated, washed repeatedly with a few milliliters of dilute H_2SO_4 and H_2O, dried over anhydrous Na_2SO_4, and evaporated. The resulting residue was dissolved in glacial AcOH (1 ml) and treated with 4 *N* HBr in glacial AcOH (1 ml). After 1 hr at 26°, Et_2O (20 ml) was added and the resulting solid material was washed several times by decantation with Et_2O. The hydrobromide salt was taken up in H_2O (0.5 ml) and the pH of the resulting solution was adjusted to 5 with 2 *N* NH_4OH. The precipitated product was washed with H_2O (0.7 ml): yield 0.065 g (72%); the compound decomposed in solid state between 230 and 270°; $[\alpha]^{21}D$ +34.9° (1% in 5 *N* HCl) [lit.[3b] $[\alpha]^{25}D$ +34.8° (1% in 1 *N* HCl)]; ir 3400 (broad NH), 1630, and 1540 cm^{-1} (carboxylate).

Anal. Calcd for $C_6H_{12}N_2O_4Se$: C, 28.2; H, 5.04; N, 10.9. Found: C, 28.5; H, 4.88; N, 11.0.

Upon amino acid analysis, L-selenolanthionine and glycine emerged at 88.5 and 105.6 ml, respectively, after the start of the chromatogram.

Elution Procedure for Amino Acids.—L-selenocystine and L-selenolanthionine were chromatographed on the Beckman–Spinco Model 120C amino acid analyzer, using Beckman custom research resin PA-28 packed in a 56 × 0.9 cm column. For elution of the amino acids a pH 3.22 ± 0.002 buffer and a pH 4.250 ± 0.002 buffer were used. Both buffers were comprised of 0.20 *N* sodium citrate containing BrIJ 35 (2.0 ml/l.), pentachlorophenol (0.1 ml/l.), and thiodiglycol reagent (5.0 ml/l.). The latter reagent was deleted during the elution of L-selenocystine.[20] The buffer flow rate was set at 68.0 ml/hr and the temperature was maintained during the entire elution procedure at 55°.

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Registry No.—II, 22423-79-6; III, 22423-80-9; IV, 22423-81-0; V, 5034-33-3; VI, 22423-83-2; VII, 22423-84-3; VIII, 22423-85-4; IX, 19641-75-9.

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Sulfur-Containing Polypeptides. X. A Study of β Elimination of Mercaptides from Cysteine Peptides[1-3]

RICHARD G. HISKEY, ROGER A. UPHAM,[4] GORDON M. BEVERLY, AND WARREN C. JONES, JR.

Venable Chemical Laboratory, The University of North Carolina, Chapel Hill, North Carolina

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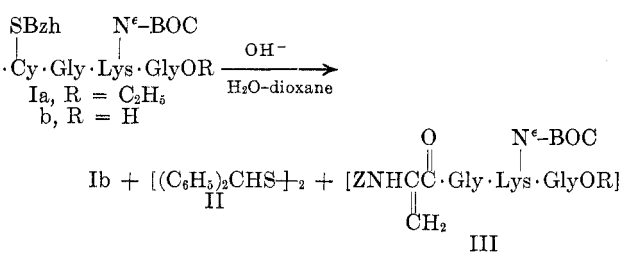
During the attempted saponification of ethyl N-carbobenzoxy-S-benzhydryl-L-cysteinylglycyl-N$^{\epsilon}$-*t*-butyloxycarbonyl-L-lysylglycinate (Ia) using 1 equiv of sodium hydroxide in aqueous dioxane, a mixture of products was obtained. In addition to some of the desired acid, Ib, the presence of several substances of higher mobility was indicated by tlc of the reaction mixture. Subsequently, one of these components was isolated and identified by melting point and mass spectral fragmentation pattern as dibenzhydryl disulfide (II). Presumably, benzhydryl mercaptide and the corresponding dehydroalanine peptide, III, were initially produced by a β-elimination[5-7] reaction; air oxidation of the mercaptide would yield II.



In order to obtain a quantitative evaluation of the extent of β elimination, a procedure devised by Patchornik and Sokolovsky[8] and Gawron and Odstrchel[9] was utilized. These workers have described an analytical method for the acid-catalyzed conversion of peptide-bound dehydroalanine to pyruvic acid and the subsequent determination of the pyruvic acid present using lactic dehydrogenase and reduced diphosphopyridine nucleotide. The amount of pyruvic acid present is assumed to represent the extent of β elimination in the original alkaline reaction. Several S-alkyl-L-cysteine esters were studied using this procedure; the substrates (Table I) included Ia, ethyl N-carbobenzoxy-S-benzyl-L-cysteinylglycinate (IV), ethyl N-carbobenzoxy-S-benzhydryl-L-cysteinylglycinate (V), and ethyl N-carbobenzoxy-S-trityl-L-cysteinylglycinate (VI). In these experiments the substrate was allowed to stand with 1.1 equiv of 1.0 *N* sodium hydroxide for 5 hr, the solvent was evaporated, and the residue was treated with 6 *N* hydrochloric acid solution at 110° for 5 hr. The amount of pyruvic acid was then determined spectrophotometrically. It is apparent from these results that the amount of β elimination is small using hydroxide ion despite the intensity of the dibenzhydryl disulfide spot on the thin layer chromatograms. The amount of pyruvic acid present was not significantly affected by solvent or the nature of the S-alkyl substituent.

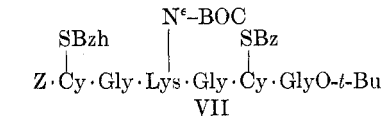
TABLE I

β ELIMINATION OF MERCAPTIDES FROM S-ALKYLCYSTEINE ESTERS DURING ESTER HYDROLYSIS

Peptide	Solvent[a]	Concn of peptide, *M*	β elimination,[b] %
Ia	EtOH	0.01	4.4, 5.0, 5.1, 5.4
Ia	DMF	0.01	4.8
IV	EtOH	0.04	4.8, 5.0, 5.3
V	EtOH	0.04	2.0, 2.3
V	DMF	0.04	2.4, 2.7
VI	EtOH	0.04	1.8
VI	DMF	0.03	1.9

[a] Solutions contain 1.1 equiv of 1.0 *N* sodium hydroxide. [b] Percentage of β elimination is equated to the percentage of pyruvic acid found in the reaction mixture after 5 hr at 25°. Pyruvic acid was determined by the procedure of A. Patchornik and M. Sokolovsky (ref 9).

The amount of β elimination accompanying the removal of an S-benzoyl group from peptides containing both S-benzhydryl- and S-benzoyl-protected L-cysteine residues was then investigated. As expected, methanolysis of *t*-butyl N-carbobenzoxy-S-benzhydryl-L-cysteinylglycyl-N$^{\epsilon}$-*t*-butyloxycarbonyl-L-lysylglycyl-S-benzoyl-L-cysteinylglycinate (VII), using dilute sodium methoxide in methanol solution (0.077 *M*), produced low levels of pyruvic acid (Table II). The hexapeptide, VII, was readily soluble in methanol, and methanolysis of the S-benzoyl group on a preparative scale proceeded rapidly and cleanly, as described by Zervas, *et al.*[10] It was noted, however, that, when the methoxide concentration was increased from 0.001 to 0.02 *M*, a spot corresponding to II appeared on the tlc of the reaction mixture. In contrast to VII, the hexapeptide, *t*-butyl N-carbobenzoxy-S-benzhydryl-



(1) Part IX of this series: R. G. Hiskey and J. T. Sparrow, *J. Org. Chem.*, **35**, 215 (1970).

(2) Supported in part by Grant A-3416 from the National Institute of Arthritis and Metabolic Diseases of the National Institutes of Health, U. S. Public Health Service.

(3) The following abbreviations have been incorporated in the text; Z = carbobenzoxy; BOC = *t*-butyloxycarbonyl; Bzh = benzhydryl; Tr = trityl; Bz = benzoyl; *t*-Bu = *t*-butyl; Cy = cysteinyl; Gly = glycyl; Lys = lysyl.

(4) Abstracted in part from a dissertation by R. A. Upham submitted to the University of North Carolina in partial fulfillment of the requirements for the Ph.D. degree, Aug 1968.

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